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Absorption and Ionization Spectra of Model Rubredoxins

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Abstract

We examine here spectroscopic properties of oxidized rubredoxin (Rdx) within the Intermediate Neglect of Differential Overlap method parametrized for spectroscopy. The Rdx structures are modeled using different length -*SR* residues bound to the Fe atom and the resulting effect on the absorption spectrum is examined. The corresponding ionization potentials are also calculated and their dependence on the molecular charge is studied. This study suggests that models used to reproduce the redox properties of these important electron-transfer compounds must be carefully chosen.

1. Introduction

We examine here the spectroscopy of model rubredoxins (Rdx), iron-sulfur proteins involved in electron-transfer and redox processes in biological systems [1,2]. Rdx systems have been extensively studied both experimentally and theoretically [3,4]. The active site of the natural protein consist of a single Fe atom, tetrahedrally coordinated by 4 sulfur-ended cysteine ligands [1,2]. X-ray structures at near atomic resolutions are available [5] as is the absorption spectrum of the protein [1,6]. The latter shows several intense bands at approximately 290, 350, 380, 490 and 580 nm which have been interpreted as ligand-to-metal charge transfer (CT) bands [6].

The oxidation state of the iron atom varies from formal charges 2+ (reduced Rdx) to 3+ (oxidized Rdx), with negative standard potential of the Fe^{+3}/Fe^{+2} couple reported as -0.57 V, contrary to the positive Fe^{+3}/Fe^{+2} potential in water +0.77 V [4]. The total number of heavy atoms varies for different proteins and is typically around 1,000 [5]. Although it is now possible to treat such molecules as a whole, using semiempirical program packages such as ZINDO [8], for example, truncation of the ligand chain is necessary especially when configuration interaction or other post SCF methods are applied.

In this work we examine the absorption spectrum and ionization potentials (IP) of rubredoxins using the Intermediate Neglect of Differential Overlap (INDO) model and examine to what extent the ligand modeling affects the theoretical results. Redox properties of these models are also discussed in light of the present calculations.

2. Computational Details

Figure 1 shows the Rdx model with the longest ligand that we examine. We assume tetrahedral S-Fe-S bond angles and adopt Fe-S bond lengths of 2.29 Å and S-C bond lengths in the thiolates of 1.80 Å, symmetrizing to the experimental X-ray structures [5].

The INDO/1 model has been used in this work as it has proven useful in a number of previous studies on transition-metal spectroscopy [8,9]. The calculations have been done within the restricted open-shell Hartree-Fock (ROHF) approximation, and subsequent configuration interaction (CI) calculations have been performed to obtain the excitation energies and the corresponding oscillator strengths. Further convolution of these bands into a Lorentzian spectrum has been done as described in detail elsewhere [9].

Calculations of the spectrum have been performed for the oxidized (Fe^{3+}) form of the model systems as the ground state geometry is known and well defined. The reduced form of the active site, Fe^{2+}, is subject to a first-order Jahn-Teller effect for both the ground and the first excited state. For this form the calculations were done for the reduced Rdx X-ray structure [5]; these latter CI calculations might be considered reliable only for vertical excitations for which the effect of the molecular distortions is minimal and can be neglected.

3. Results and Discussion

The absorption spectra we calculated are summarized in Table 1 and displayed in Figures 2 and 3. As seen from Table 1, the excitation energies derived via ROHF-CIS drop significantly with the increase of the thiolate chain, the best result being obtained with

the longest ligand SCH_2CH_3. Further enlargement of the aliphatic chain, or introducing peptide links into it, does not produce a markable decrease of the CIS energies (data not shown). The same trend is observed with the more computer demanding CISD calculations which, in turn, are seen to improve the results when compared to the lowest transition observed in the native Rdx at 17,200 cm^{-1} [6].

The energy drop in the series SH - SCH_3 - SCH_2CH_3 can be related to the lower energy IPs of the ligand, and these, in turn, lower the L→M CT-energies. The corresponding ligand IPs [7] are shown in Table 2 where they are compared with the calculated values for different model ligands utilized in this work. One can observe the trend of diminishing the IPs with extending the thiolate chain, reaching saturation at about the n-thiopropyl. These energies are well reproduced by our calculations, at the SCF level, Table 2, and then slightly improved using the CIS method. The molecular orbital (MO) analysis shows that the IP excitations take place from highest occupied MO (HOMO) which is dominated by sulfur *3p* atomic orbital (AO) contributions, a contribution that decreases as the aliphatic chain becomes longer. This process is accompanied by increase of the carbon atoms *2p* orbital contribution which becomes dominant in the HOMO for the longest ligand studied. However, even though the HOMO is dominated by carbon orbitals, ΔE(SCF) calculations indicate that in all cases the actual excitation takes place from an effective orbital which is sulfur dominated. This process is displayed in Figure 4 and can be explained by the larger relaxation of the sulfur orbitals compared to that of the carbon ones.

Ionization energies in these model rubredoxins are important quantities with respect to the electronic structure simulation of the natural protein. Table 3 reports the total

energy as a function of the charge n of the model $Fe(SCH_2CH_3)_4^n$ compound. The n=-1 compound is calculated to be the most stable, formally Fe^{3+} as observed. A negative IP (more stable oxidized form) is found for the Fe^{3+} model in agreement with the reductive potential of the protein, and then the IPs increase almost linearly with the charge. The first IP 6.23 eV is in good agreement with most estimates of *3d* transition-metal IPs for such complexes ranging from 6.8 to 7.6 eV. The added charge in the Fe^{+3} to Fe^{+2} transition, goes, however, mostly to the ligand orbitals as can be seen from the Mulliken population analysis of the above molecules, Table 4.

The measured 1-electron oxidation potential of Rdx is -0.57 V, in good agreement with our value of -0.19 eV, Table 3. This redox potential is generally attributed to Fe^{3+}/Fe^{2+}, although we see from Table 4 that most of the added charge goes to the ligands. The final d-electron count, however, does suggest $Fe^{3+} + e \rightarrow Fe^{2+}$. The experimental atomic IP of Fe^{2+} is reported at 30.638 eV. We calculate this value 25.387 eV.

We might estimate the redox potential of the Fe^{+3}/Fe^{+2} couple in the protein from the self-consistent reaction field theory [10-12], the first term of which is the Born term

$$E = -q^2(1 - \frac{1}{\epsilon})/2a_o \tag{1}$$

where E is in Hatrees (1 Hartree = 27.21 eV), ϵ is the dielectric constant (dimensionless, 80 for water, 10 for a structured protein), and a_o is a cavity radius.

We estimate an effective cavity radius of the Fe atom in the complex from the mass density

$$\frac{4\pi a_o^3}{3} = \frac{mm}{\rho N_A} \tag{2}$$

where ρ is density, N_A is ithe Avogadro's number, mm is the molecular mass [12].

This yields, in Bohr,

$$a_\circ = 1.387(\frac{mm}{\rho})^{\frac{1}{3}} \tag{3}$$

For ρ=7.87 g/cm^3 for Fe, $a_\circ$=2.66 Bohrs (1.408 Å). Assuming ϵ=10, a value commonly used for a structural protein, we estimate an energy correction for the q=3+ to q=2+ process of -26.06 eV. Adding this to our calculated atomic value of 25.39 eV yields -0.67 V for the Fe^{+3}/Fe^{+2} couple in the model Rdx, remarkably close to the -0.19 eV value we calculate for the molecule itself, given the simplicity of the model used.

This simple calculation demonstrates that the ligands are specifically designed to adjust the Fe^{+3}/Fe^{+2} redox potential to that required in the chemistry of specific systems.

On the basis of these results we can conclude that proper ligand modeling is essential to correctly reproduce the electronic structure of iron-sulfur proteins. Although this is, perhaps, not a surprising result, it is a cautionary one in that simpler ligands may not be appropriate. We are also given some confidence in these calculations and their agreement with experiment that the INDO model, itself, is useful in the study of iron-sulfur proteins.

Acknowledgment

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Table 1 Calculated lowest excitation energies (cm^{-1}) for model rubredoxins. CIS and CISD indicate CI singles, and CI singles and doubles methods, respectively. The lowest experimental energy is at 17,200 cm $^{-1}$, Ref. 6.

Model	Energy	CI type
$Fe(SH)_4$	25,300	CIS
$Fe(SCH_3)_4$	23,400	CIS
$Fe(SCH_2CH_3)_4$	21,300	CIS
$Fe(SCH_2CH_3)_4$	19,000	CISD

Table 2 Calculated and experimental vertical ionization potentials for $SC_{2n}H_{2n+2}$, n=0-3 [E. Gallegos and R.W. Kiser, *J. Phys. Chem.* 65 (1961) 1177] and the HOMO energy (eV). First column of the calculated values (this work) presents the SCF energies, second column - CIS energies. Numbers in parentheses indicate the two highest AO contributions to the HOMO.

Compound	Experiment	This work		HOMO(S_{3p},C_{2p})
HSH	10.47	10.31	10.29	-10.81(1.00,0.00)
$HSCH_3$	9.44	9.52	9.50	-10.00(0.72,0.15)
$HSCH_2CH_3$	9.29	9.51	9.49	-9.98(0.35,0.21)
$HSCH_2CH_2CH_3$	9.20	9.54	9.51	-9.18(0.00,0.46)
				-10.18(0.89,0.03)

Table 3 Calculated ionization energies (eV) for the $Fe(SCH_2CH_3)_4^n$ model rubredoxin. SCF energies in Hartrees.

Net Charge, n	2S+1	SCF energy	E_n-E_{n-1}
-2	5	-115.8245	-
-1	6	-115.8313	-0.0068
0	5	-115.6022	0.2291
+1	4	-114.2608	0.3414
+2	3	-114.7323	0.5285
+3	2	-114.1089	0.6234
+4	1	-113.3688	0.7401

Table 4 Charge distribution within $Fe(SCH_2CH_3)_4^n$ according to the Mulliken population analysis.

Net Charge, n	Fe	S(1-4)	C(1-4)'	C(1-4)"	H(1-20)
-1	1.38	-0.63	-0.13	-0.04	0.01
-2	1.18	-0.76	-0.15	-0.05	0.01

Fig. 1

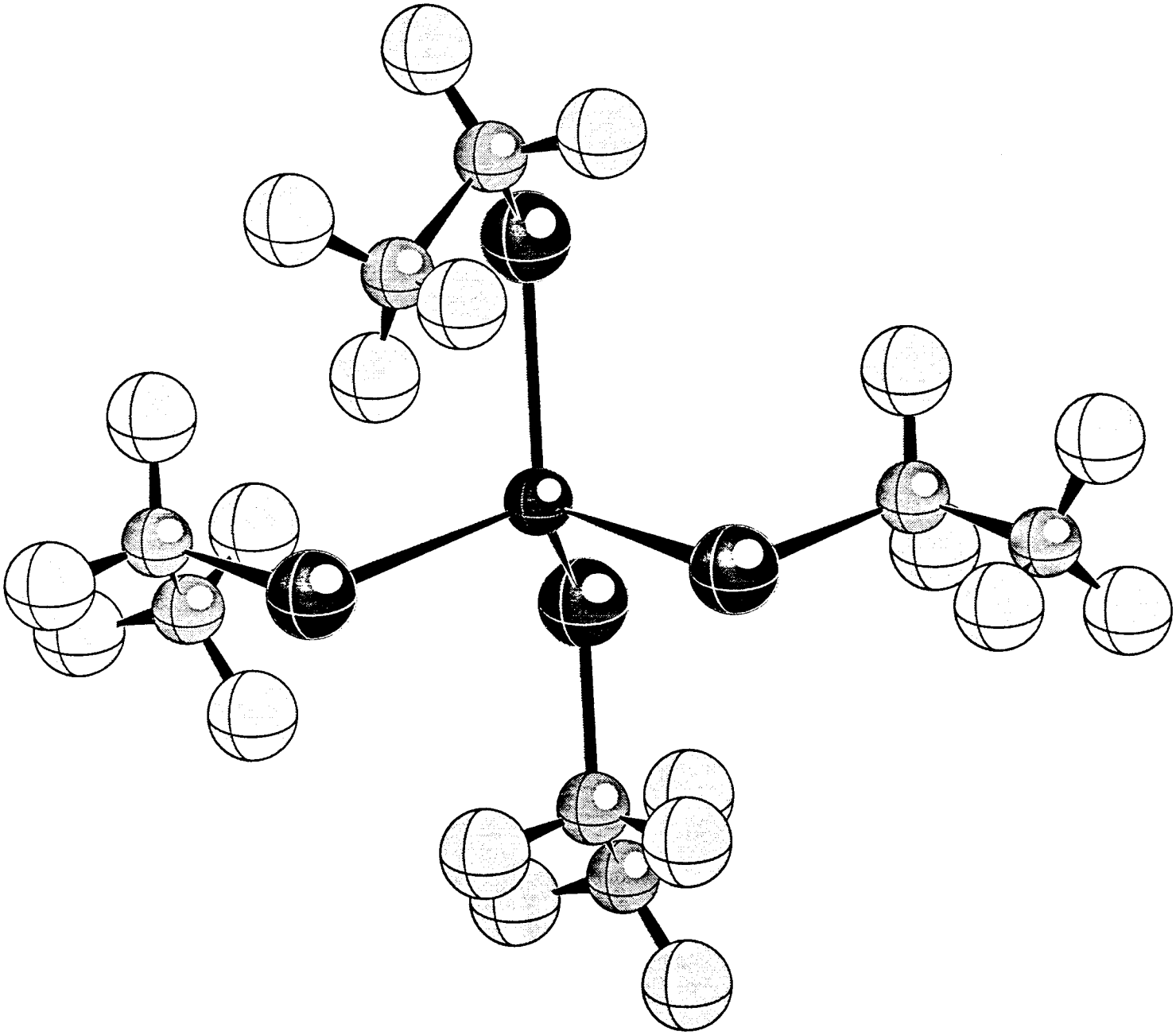
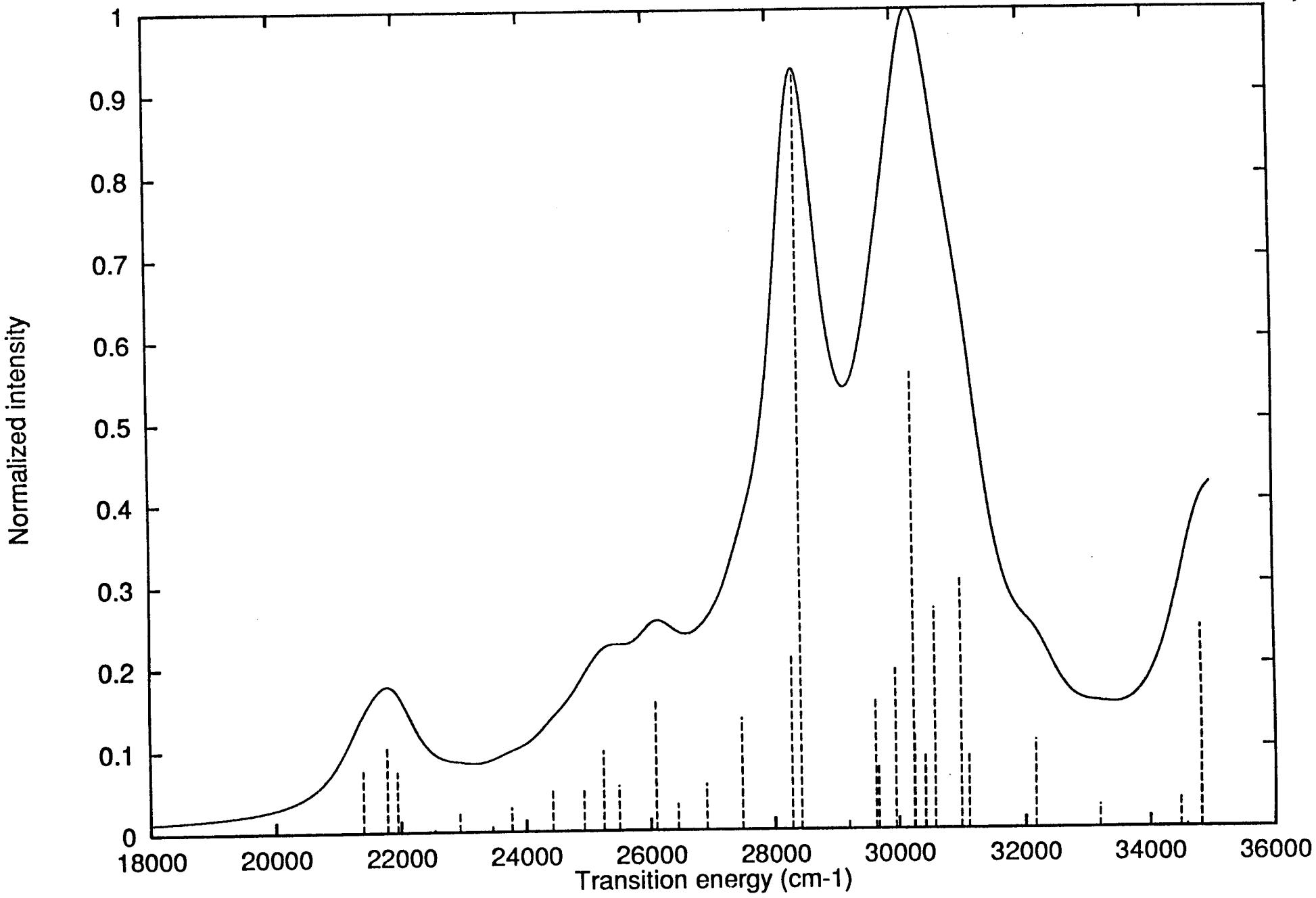


Fig. 2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0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
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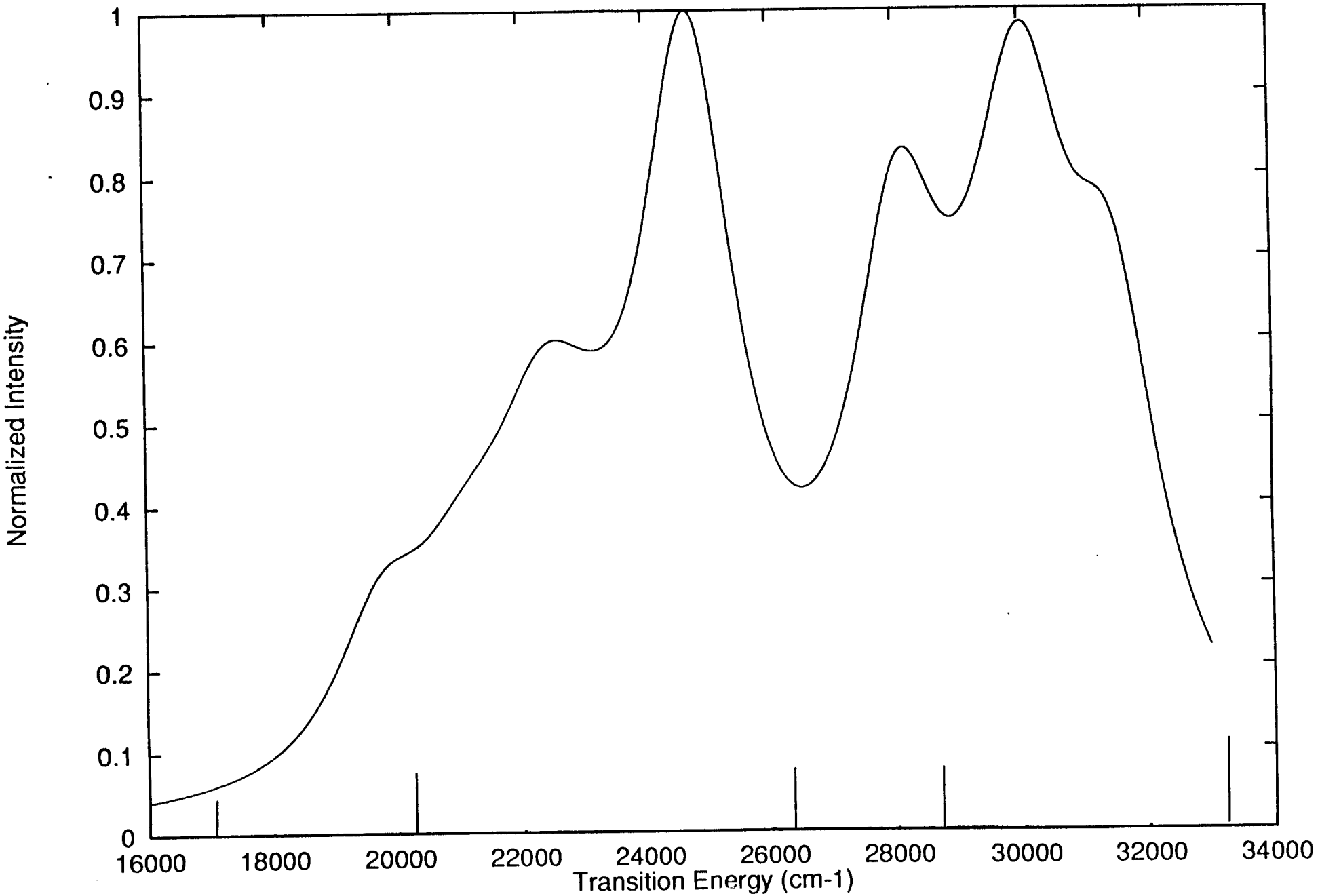
20000
22000
24000
26000
28000
30000
32000
34000
36000
Transition energy (cm-1)

Fig. 3


Normalized Intensity
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0.7
0.6
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0.4
0.3
0.2
0.1
0
16000
18000
20000
22000
24000
26000
28000
30000
32000
34000
Transition Energy (cm-1)

Figure Captions

Figure 1 The model $Fe(SCH_2CH_3)_4^-$ structure of 1-Fe rubredoxin.

Figure 2 Lorentzian spectrum convoluted for the $Fe(SCH_2CH_3)_4^-$ model (Fig.1) from the CIS calculated values. The calculated energies appear as dashed vertical lines with heights proportional to the oscillator strengths.

Figure 3 Lorentzian spectrum convoluted for the $Fe(SCH_2CH_3)_4^-$ model (Fig.1) from the CISD calculated energies compared with the experimental band positions (solid vertical lines).